REVIEW

REVISED Control of social status by sex steroids: insights from teleost fishes

[version 2; peer review: 2 approved]

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Abstract

Many animals live in highly social environments, in which individuals must behave in a way that enables them to survive and live harmoniously among conspecifics. Dominance hierarchies are typical among social species and are essential for determining and preserving stability within social groups. Although there is considerable evidence that sex steroid hormones regulate behaviors associated with dominance, such as aggression and mating, fewer studies have examined the role of these hormones in controlling social status, especially in species that exhibit social hierarchies. Furthermore, despite this research, we know remarkably little about the precise neural and molecular mechanisms through which sex steroids modulate traits associated with social rank. Here, we review the neuroendocrine regulation of social status by sex steroids in teleost fishes, the largest and most diverse vertebrate group that shows extensive variation in reproductive systems and social structures between species. First, we describe the functions of sex steroids and novel steroid-related genes that teleost fishes possess due to a lineage-specific whole-genome duplication event. Then, we discuss correlational, pharmacological, and molecular genetic studies on the control of social status by sex steroids in teleost fishes, including recent studies that have implemented gene editing technologies, such as CRISPR/Cas9. Finally, we argue that gene editing approaches in teleost studies, within both integrative and comparative frameworks, will be vital for elucidating the role of sex steroids in controlling social rank and characterizing their neural and molecular mechanisms of action. Collectively, ongoing and future research in these species will provide novel insight into the evolution of the regulation of social status by sex steroids and other neuroendocrine substrates across vertebrates.

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Any reports and responses or comments on the article can be found at the end of the article.

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REVISED **Amendments from Version 1**

We have updated the article in response to both reviewers' concerns and suggestions. This includes updating our references and clarifying arguments regarding the role of androgens in social behavior.

Any further responses from the reviewers can be found at the end of the article

1. Introduction

Hierarchies are a common feature among social organisms[1–3]. Rank along a social hierarchy impacts which members of a group have access to shelter, mates, and other resources, such as food. While social hierarchies can vary considerably in size and composition, they generally consist of higher-ranking dominant (DOM) individuals, which tend to have greater access to resources and more opportunities for mating compared to lower-ranking subordinate (SUB) individuals[2,4,5]. Rank within a hierarchy is determined by frequent social interactions among individuals and is associated with distinct physiological and behavioral traits[6,7]. These hierarchical structures, however, are dynamic and can be altered due to naturally occurring changes in the social environment (e.g., predation, death). Thus, in many species, individuals may have the opportunity to rise in social rank (i.e., social ascent) or descend in social rank (i.e., social descent). In addition to enhancing social stability, social hierarchies can have both positive and negative impacts on mental and physical health[2]. The influence of social and environmental cues on social rank has been studied extensively in a variety of vertebrate species, including mice[8,9], rats[10–13], fishes[14], lizards[15–17], and primates[2]. Variation in numerous species-specific traits that are modulated by the social environment influences an individual's social rank, such as body size, social behaviors, and territory ownership. Well-known physiological correlates of social rank include a positive association between sex steroid hormone levels (e.g., androgens and estrogens), gonad mass, and territorial and mating behaviors.

Despite what is known about the behavioral and physiological traits linked with social rank, the molecular and neural mechanisms governing social status are unclear. The mechanisms controlling DOM-typical social behaviors (e.g., aggression, mating), however, have been investigated in detail. These studies have shown that androgens and estrogens control aggression and mating behaviors across vertebrate species[18,19]. Moreover, there is considerable evidence that steroid hormone levels differ with social status and that correlations between steroids and behaviors associated with social rank are stronger during periods of social instability (e.g., establishment of dominance hierarchies or territories, competition)[20–23]. Fewer studies have characterized the neural and molecular mechanisms that regulate social status specifically, although recent work has begun to shed light on some of the processes involved[24–26]. Given the strong link between sex steroid hormones and social rank across vertebrates, investigating how sex steroid signaling systems control social status is warranted. Additionally, sex steroid receptors and their ligands have relatively high homology among vertebrates[27,28] and follow conserved expression patterns in the brain[18,29,30]. Therefore, studying the regulation of social status by sex steroid hormones within a comparative framework may lead to important discoveries on the fundamental mechanisms underlying key aspects of social status across species. One of the most diverse groups of species for which comparative work may be especially fruitful are teleost fishes. In this review, we highlight research that has examined the relationships between sex steroid signaling and social status in teleosts. Given the diversity of life histories, species-specific variations in social hierarchies, and the availability of state-of-the-art genetic tools among these species, teleost fishes are an excellent group for enhancing our understanding of the control of social status by sex steroids. We begin our review by briefly describing the general functions of steroid hormones and the unique steroid signaling genes that teleosts possess due to a lineage-specific whole-genome duplication. Then, we discuss correlational, pharmacological, and genetic studies that provide insight into the regulation of social status by sex steroid hormones in teleost fishes. We assert that the use of teleost fishes as model organisms, along with the integration of gene editing methodologies, will be critical for elucidating the functions of sex steroids in governing social status across species.

2. A primer on steroid hormones in teleost fishes

2.1. Brief background on steroid mechanisms of action

Steroid hormones are a group of lipophilic molecules with a cholesterol backbone that can act as transcription factors or secondary messengers by binding to membranous, cytoplasmic, or nuclear receptors[31]. Steroidogenesis occurs in peripheral tissues, such as the gonads (the primary source of sex steroids) and adrenal glands, and specific brain regions through the actions of enzymes that synthesize steroids *de novo* from cholesterol or from prohormones in circulation[32]. Prohormones are often found at high levels in the blood and have lower affinity for receptors compared to their more active metabolites[33,34]. Thus, the conversion of prohormones to more potent steroids within target tissues is critical for modulating physiological processes[35]. For example, the androgen testosterone (T) can be converted to both androgenic and estrogenic metabolites, such as the androgen 5α-dihydrotestosterone via the enzyme 5α-reductase; the androgen 11-ketotesteosterone (11-KT) via the enzymes 11-hydroxysteroid dehydrogenase types 1 and 2, which is the main androgen in teleost fishes and is also synthesized by other vertebrates[36–38]; and the estrogen 17β-estradiol (E_2) via the enzyme aromatase[35,36].

Steroid receptors modulate a suite of processes, such as cellular signaling, body temperature and other homeostatic functions, sexual differentiation, and social behaviors. These processes are controlled through steroid receptors acting via one of two mechanisms: 1) a "genomic" mechanism, in which steroids bind to cytoplasmic or nuclear receptors, and 2) a "non-genomic" mechanism, in which steroids activate a second messenger cascade by binding to G-protein coupled receptors or by modifying membrane-located ionic receptors[39–41]. For example, testosterone can exhibit genomic mechanisms of action by binding to the androgen receptor, a process that results in dimerization and the formation of an androgen-receptor complex that then binds to a nuclear receptor to modulate gene expression. Conversely, non-genomic mechanisms of action may lead to indirect changes in gene expression, depending on the secondary messenger system or specific ion channels that are activated. Collectively, there are five major classes of steroid receptors, including androgen receptors (ARs), estrogen receptors (ERs), glucocorticoid receptors (GRs), mineralocorticoid receptors (MRs), and progesterone receptors (PRs). Like steroidogenic enzymes, steroid receptors are expressed in the periphery and brain.

2.2. Teleosts possess novel steroid receptors resulting from a teleost-specific whole-genome duplication

About 350 million years ago, the common ancestor of all teleost fishes underwent a whole-genome duplication event[42,43]. This teleost-specific whole-genome duplication (TS-WGD) led to the replication of many important genes, including those associated with steroidogenesis and steroid signaling. After a whole-genome duplication event, duplicate genes (also referred to as "paralogs") may face one or more of several fates. Most commonly, a redundant gene experiences relaxed selective pressure, which leads to the buildup of deleterious mutations and culminates in non-functionalization[44]. Alternatively, a duplicate gene may undergo neofunctionalization, in which a gene experiences positive selection due to the accumulation of mutations that confer novel functions[44,45]. In some cases, both gene paralogs may be maintained due to the partitioning of ancestral functions between the two daughter genes, a process referred as subfunctionalization[45,46].

Many of the novel duplicate genes that encode steroidogenic enzymes and steroid receptors have been retained in teleost fishes, which may have a profound impact on the diversity of reproductive strategies and social structures in this lineage[47–51]. While all other vertebrates possess one AR encoded by a single gene, most teleost fishes express two distinct ARs (ARα and ARβ), which are encoded by two different genes (*ar1* and *ar2*)[19]. Furthermore, teleosts possess extra copies of ERs. Like other vertebrates, teleosts possess ERα and ERβ, which are encoded by the genes *esr1* and *esr2*, as well as an additional copy of ERβ. Therefore, teleosts have two ERβs (ERβ1 and ERβ2), which are encoded by *esr2a* and *esr2b,* respectively. Numerous teleost species also possess an extra GR and, thus, have two GRs (GRα and GRβ), which are encoded by the genes *nr3c1a* and *nr3c1b*, respectively. Conversely, teleost fishes have only one MR. Most teleosts only have one PR, but goldfish (*Carassius auratus*) have two PRs (PRα and PRβ) that are encoded by distinct genes (*pgr1* and *pgr2*), suggesting that the duplicate PRs were retained following the TS-WGD in this species. As observed for steroid receptors, teleost fishes also possess duplicate genes of many steroidogenic enzymes. For example, in teleosts, the two aromatase genes, *cyp19a1* and *cyp19a1a*, are highly conserved and contrast with all other vertebrates, which only possess one aromatase gene. To date, the functions of these steroid systems in regulating social behaviors and social status have primarily been examined using correlational and pharmacological approaches. Thus, additional studies that use genetic manipulations to understand how steroids control social status and its associated physiological and behavioral processes are needed.

3. Neuroendocrine control of social status by sex steroids in teleosts

Many teleost fishes live in social groups, in which stability is maintained via hierarchies in one or more sexes[52,53]. In this section, we discuss themes and variations that have been revealed about the role of steroid hormones in modulating behaviors associated with social status through studies of teleost fishes, specifically by highlighting correlational, pharmacological, and genetic studies of the neuroendocrine regulation of social status by steroids. We focus our review on sex steroids (androgens, estrogens, and progestins) as modulators of DOM-typical behaviors (e.g., aggression, mating), which have been major emphases of research investigating how steroids control social status in teleosts for the past several decades (for more information on the regulation of social status by glucocorticoids in teleost fishes and other vertebrates, see these reviews: 54–57).

3.1. Associations between sex steroids and social rank

The androgens T and 11-KT have been extensively studied for their functions in regulating aggression and mating in teleosts, including species that exhibit social hierarchies (Table 1)[22,23]. Numerous studies have shown that DOM males have higher circulating androgen levels than SUB males, including African cichlids [Burton's mouthbrooder (*Astatotilapia burtoni*)[58–62]; Mozambique tilapia (*Oreochromis mossambicus*)[63]; Nile tilapia (*Oreochromis niloticus*)[64]; daffodil cichlid (*Neolamprologus pulcher*)[65–67]; Dimerus cichlid (*Cichlasoma dimerus*)[68,69]; Nyerere's Victoria cichlid (*Pundamilia nyererei*)[70]], salmonids [rainbow trout (*Oncorhynchus mykiss*)[71,72]; brown trout (*Salmo trutta*)[71]; Arctic char (*Salvelinus alpinus*)[73]], reef fish [stoplight parrotfish (*Sparisoma viride*)[74]; New Zealand demoiselle (*Chromis dispilus*)[75]; ocellated wrasse (*Symphodus ocellatus*)[76]], Japanese medaka (*Oryzias latipes*)[77], and zebrafish (*Danio rerio*)[78]. Moreover, levels of DOM-typical behaviors, such as male-male aggression and reproductive behaviors, are positively associated with circulating T and/or 11-KT in some of these species (e.g., *A. burtoni*[79,80]; *C. dimerus*[81]). Similar relationships between circulating androgen levels and aggressive and reproductive behaviors have been documented in some teleost species that do not readily form social hierarchies, including Siamese fighting fish (*Betta splendens*)[82,83], peacock blennies (*Salaria pavo*)[84,85], swordtail fish (*Xiphophorus helleri*)[86], white perch (*Morone americana*)[87], Gulf toadfish (*Opsanus beta*)[88], plainfin midshipman (*Porichthys notatus*)[89], and three-spined stickleback (*Gasterosteus aculeatus*)[90,91]. Few studies have sought to distinguish how circulating 11-KT and T are linked with aggressive and reproductive behaviors in teleosts, but work in African cichlids suggests that both

Table 1. Summary of correlational, pharmacological, and gene editing studies in teleost fishes that suggest a role for sex steroids in regulating social status in males.

Scientific Name	Common Name	Family	Hierarchy	Steroid	Circulating Levels	Pharmacology	Gene Editing	References
Astatotilapia burtoni	Burton's mouthbrooder	Cichlidae	♂ only	T	DOM > SUB	T ↑ aggression; DHT ↑ courtship; CA ↓ courtship	ARα mutation ↓ courtship and aggression; ARαβ mutation ↓ aggression	Alward *et al.*, 2019[58], 2020[26]; Fernald, 1976[92]; Maruska and Fernald, 2010[59]; Maruska *et al.*, 2013[60]; O'Connell *et al.*, 2013[61]; O'Connell and Hofmann, 2012[93]; Parikh *et al.*, 2006[62]
				11-KT	DOM > SUB	---	---	Alward *et al.*, 2019[58]; Maruska and Fernald, 2010[59]; Maruska *et al.*, 2013[60]; Parikh *et al.*, 2006[62]
				E_2	DOM > SUB	E_2 ↑ aggression; FAD ↓ aggression	N/A	Huffman *et al.*, 2013[94]; Maruska and Fernald, 2010[59]; Maruska *et al.*, 2013[60]; O'Connell *et al.*, 2013[61]; O'Connell and Hofmann, 2012[93]
				PROG	DOM > SUB	DHP ↑ courtship; ZK0112993 ↓ courtship	N/A	O'Connell *et al.*, 2013[61]; O'Connell and Hofmann, 2012[93]
Oreochromis mossambicus	Mozambique tilapia	Cichlidae	♂ only	11-KT	DOM > SUB	---	---	Golan and Levavi-Sivan, 2013[63]
Oreochromis niloticus	Nile tilapia	Cichlidae	♂ only	11-KT	DOM > SUB	---	---	Pfennig *et al.*, 2012[64]
Pundamilia nyererei	Nyerere's Victoria cichlid	Cichlidae	♂ only	11-KT	DOM > SUB	---	---	Dijkstra *et al.*, 2007[70]
Cichlasoma dimerus	Dimerus cichlid	Cichlidae	Sex-specific (♂ only and ♀ only)	T	DOM > SUB	N/A	N/A	Ramallo *et al.*, 2015[69]
				11-KT	DOM > SUB	---	---	Morandini *et al.*, 2014[68]; Ramallo *et al.*, 2015[69]
				E_2	DOM < SUB	N/A	N/A	Ramallo *et al.*, 2015[69]
Neolamprologus pulcher	Daffodil cichlid	Cichlidae	All individuals (♂ and ♀)	T	DOM > SUB	N/A	N/A	Aubin-Horth *et al.*, 2007[65]; Desjardins *et al.*, 2008[66]
				11-KT	DOM > SUB	---	---	Desjardins *et al.*, 2008[66]; Taves *et al.*, 2009[67]
Oncorhynchus mykiss	Rainbow trout	Salmonidae	♂ only	T	DOM > SUB	N/A	N/A	Cardwell *et al.*, 1996[71]; Liley and Kroon, 1995[72]
				11-KT	DOM > SUB	---	---	Liley and Kroon, 1995[72]

Scientific Name	Common Name	Family	Hierarchy	Steroid	Circulating Levels	Pharmacology	Gene Editing	References
Salmo trutta	Brown trout	Salmonidae	♂ only	T	DOM = SUB	N/A	N/A	Cardwell *et al.*, 1996[71]
				11-KT	DOM > SUB	---	---	Cardwell *et al.*, 1996[71]
Salvelinus alpinus	Arctic char	Salmonidae	♂ only	T	DOM > SUB	N/A	N/A	Elofsson *et al.*, 2000[73]
				11-KT	DOM > SUB	---	---	Elofsson *et al.*, 2000[73]
Sparisoma viride	Stoplight parrotfish	Scaridae	♂ only	T	DOM > SUB	N/A	N/A	Cardwell and Liley, 1991[74]
				11-KT	DOM > SUB	---	---	Cardwell and Liley, 1991[74]
Chromis dispilus	New Zealand demoiselle	Pomacentridae	♂ only	T	DOM > SUB	N/A	N/A	Pankhurst and Barnett, 1993[75]
				11-KT	DOM > SUB	---	---	Pankhurst and Barnett, 1993[75]
Symphodus ocellatus	Ocellated wrasse	Labridae	♂ only	T	DOM = SUB	N/A	N/A	Stiver *et al.*, 2015[76]
				11-KT	DOM > SUB	---	---	Stiver *et al.*, 2015[76]
				E_2	DOM = SUB	N/A	N/A	Stiver *et al.*, 2015[76]
Oryzias latipes	Japanese medaka	Adrianichthyidae	♂ only	T	N/A	N/A	ARα mutation ↓ reproductive behavior; ARβ mutation ↓ sexual motivation	Ogino *et al.*, 2023[95]
				11-KT	DOM > SUB	---	---	Kagawa *et al.*, 2017[77]
				E_2	N/A	E_2 and EE2 ↓ courtship	N/A	Balch *et al.*, 2004[96]; Oshima *et al.*, 2003[97]
Danio rerio	Zebrafish	Cyprinidae	Sex-specific (♂ only and ♀ only)	T	N/A	BPA ↓ courtship and ↑ aggression	AR KO ↓ courtship and aggression	Carver *et al.*, 2021[98]; Lu *et al.*, 2017[99]; Yong *et al.*, 2017[100]
				11-KT	DOM > SUB	---	---	Filby *et al.*, 2010[78]
				E_2		EE2 ↑ aggression and ↓ social preference	N/A	Fenske *et al.*, 2020[101]
				PROG	N/A	N/A	PR KO ↑ aggression	Carver *et al.*, 2021[98]

Results of studies that measured circulating sex steroid levels or the effects of pharmacological manipulations or genetic deletion of sex steroid receptors on dominant-typical behaviors in teleosts. Findings that are reported in this table for pharmacological studies are from dominant males. Only studies that investigated these mechanisms in teleost species that form social hierarchies are shown (see Section 3 for additional studies that examined the role of sex steroids in regulating aggressive or mating behaviors in males of species that do not form social hierarchies). *Abbreviations: 11-KT, 11-ketotestosterone; ARα, androgen receptor alpha; ARβ, androgen receptor beta; BPA, bisphenol A; CA, cyproterone acetate; DHT, 5α-dihydrotestosterone; DHP, 5α-dihydroprogesterone; DOM, dominant;* E_2*, 17β-estradiol; EE2, ethinyl estradiol; FAD, fadrozole; KO, knockout; MT, 17α-methyltestosterone; PROG, progesterone; SUB, subordinate; T, testosterone.*

of these androgens generally exhibit positive relationships with aggression[62,67]. Collectively, these studies suggest a conserved role of androgens in controlling DOM-typical behaviors (e.g., aggression, mating) in teleost fishes. Further work is necessary, however, to determine whether 11-KT and T have distinct functions in regulating aggressive and reproductive behaviors, a question that can be addressed using pharmacological or molecular genetic approaches to selectively target their synthetic pathways.

In addition, androgens can be influenced by the social environment, even within a given social rank, which can affect the organization and stability of a dominance hierarchy within a population[102–104]. For example, DOM males exhibit an increase in circulating T and/or 11-KT following inter-male agonistic interactions in many teleost species [*A. burtoni*[59]; *N. pulcher*[67,105]; *O. mossambicus*[106,107]; *P. nyererei*[70]; callipterus cichlid (*Lamprologus callipterus*), blunthead cichlid (*Tropheus moorii*), and *Pseudosimochromis curvifrons*[108]; *D. rerio*[107,109]]. Moreover, higher baseline levels of circulating androgens in DOM males can reinforce their status by increasing their chances of winning future agonistic interactions, a phenomenon called the winner effect (*O. mossambicus*[106]; *Pundamilia* sp.[110]). Prior work also suggests that androgens are altered by social context, including exposure to other social interactions or environmental cues that may precede subsequent territorial intrusions (i.e., the bystander effect; *O. mossambicus*[111,112]), the presence of conspecifics during an agonistic encounter (i.e., audience effects; *O. mossambicus*[113]; *B. splendens*[82]), and the familiarity of an opponent (i.e., the dear enemy effect; *O. mossambicus*[114]). Together, these studies demonstrate how circulating T and 11-KT are affected not only by social status, but also by the social interactions that individuals encounter in their environment.

Although few studies have examined how estrogens and progestins vary based on social status, there is some support that these hormones are linked with a DOM social rank and its associated behaviors in cichlids[22]. Circulating levels of E_2 and progestins are higher in DOM males than SUB males in *A. burtoni*[61,115]. Furthermore, E_2 and progestins are positively correlated with aggressive and reproductive behaviors in DOM male *A. burtoni*[80]. It is important to note, however, that similar relationships between estrogens, progestins, and social status are not exhibited by all cichlids. For example, DOM male *C. dimerus* have lower circulating E_2 than SUB males, yet DOM males increase plasma E_2 levels following an aggressive interaction[69,116]. Collectively, these findings suggest that circulating estrogens and progestins are linked with social status in cichlids, including aggression and mating behaviors, but that the direction of these correlations are species-specific. Given the paucity of research on how circulating estrogens and progestins are associated with social status and aggressive and reproductive behaviors, further studies are necessary to investigate these potential relationships in other teleost species, including those that exhibit and do not exhibit dominance hierarchies.

3.2. Pharmacological studies to understand the regulation of social status by sex steroids

In several teleost species, pharmacological manipulations have been used to investigate the role of sex steroid hormones in controlling aggressive and mating behaviors. Much of this work utilizes drugs that modify the activity of ER, AR, or PR signaling. A number of studies in zebrafish have used synthetic estrogen analogs to assess their impact on behavior, whereas others have used AR and PR agonists and antagonists to determine the role of these receptor systems in regulating aggressive and mating behaviors. In addition, the functions of sex steroids in modulating aggression, mating, and social status have been studied in-depth in *A. burtoni*, our lab's study species. In this section, we summarize pharmacological studies that have been conducted across teleost species to understand the regulation of social status by sex steroids, with an emphasis on findings in *A. burtoni*.

Zebrafish aggressive and mating behaviors have been studied in detail, and most pharmacological findings on the role of steroid hormones in regulating aggression and mating in this species are from experiments performed within a toxicology framework[117,118]. For example, the effects of the synthetic estrogen analog ethinyl estradiol (EE2), a main ingredient in most birth control pills, and Bisphenol A (BPA), an endocrine disrupting chemical found in industrial plastics, have been tested in male and female zebrafish. BPA has been shown to act as an ER agonist, an AR antagonist, and an inhibitor of T synthesis[119]. In male zebrafish, EE2 treatment enhances aggression in a mirror assay and reduces social preference in a social cohesion assay[101], whereas BPA administration reduces courtship behavior, but increases aggression towards male conspecifics[120]. Similar results have been found in medaka species: E_2 injections and EE2 treatment significantly reduce mating behavior in male Japanese medaka[96,121], and EE2 administration decreases reproductive behaviors in mating pairs of brackish medaka (*Oryzias melastigma*)[121]. Similar to findings in medaka and zebrafish, EE2 treatment disrupts reproductive and aggressive behaviors in male fathead minnows (*Pimephales promelas*)[122]. Males given EE2 show a reduced ability to compete for and clean spawning sites and exhibit a lower frequency of chasing behavior directed towards male conspecifics.

Further insight into the neuroendocrine regulation of aggression and mating has been gained from research that has altered androgen levels using synthetic compounds. For example, Belanger *et al.* tested the effects of the aromatizable synthetic androgen 17α-methyltestosterone (MT) on sensitivity to female pheromones and courtship behaviors in males of four different cyprinid species: tinfoil barbs (*Barbonymus schwanenfeldii*), redtail sharkminnows (*Epalzeorhynchos bicolor*), goldfish, and zebrafish[123]. MT injections enhanced pheromone detection of prostaglandins (measured via electroolfactogram recordings) in all four species and elevated courtship in juvenile redtail sharkminnows. These results suggest that cyprinids share evolutionarily conserved olfactory, but not behavioral responses to pheromonal prostaglandins, mechanisms that are mediated by androgens.

Moreover, the use of steroid agonists and antagonists has been critical for examining how sex steroids influence DOM-typical behaviors. In the monogamous convict cichlid (*Amatitlania nigrofasciata*), treatment with the non-steroidal AR antagonist flutamide reduces courtship behaviors, but has no effect on aggressive behaviors in males[124]. In male *A. burtoni*, injection of the aromatase inhibitor fadrozole (FAD) reduces the frequency of attacks directed towards male opponents, but does not affect courtship[94]. Likewise, FAD treatment decreases the rate of attacks directed towards male conspecifics in the weakly electric fish *Gymnotus omarorum*[125]. Therefore, results from *A. burtoni* and weakly electric fish demonstrate that estrogen synthesis is required for aggressive, but not courtship behaviors. These findings contrast with those described above for the effects of the synthetic estrogen EE2 on behavior in zebrafish and medaka, which could suggest that EE2 administration affects certain estrogenic signaling pathways differently than naturally synthesized estrogens. Nonetheless, the diversity of teleost fishes in which the influence of estrogenic signaling molecules can be studied, together with an array of available pharmacological approaches, will yield fundamental discoveries on the hormonal mechanisms underlying social behaviors.

In addition to variation in reproductive systems, teleost fishes also exhibit diverse parental care strategies, providing further avenues for exploring the control of mating and aggressive behaviors by steroid hormones across species using pharmacological manipulations. For example, in bluegill (*Lepomis macrochirus*), parental behavior is exhibited solely by males. To test how the neuroendocrine control of mating behavior may be affected in this system, Kindler *et al.* tested the effects of two androgens, 11-KT and T, and cyproterone acetate (CA), a steroidal AR antagonist, on courtship behavior in male bluegill during prespawning and parental periods[126]. Using CA to block AR function may be ideal in teleost species, given the presence of an additional, novel AR paralogous gene. Indeed, since CA is steroidal, it blocks access of androgens to the ligand binding domains of either AR[127]. 11-KT and T implants failed to stimulate nest building in parental males in spring and early summer, while CA treatment reduced reproduction in male bluegill. 11-KT treatment did, however, enhance courtship behaviors in nesting males, whereas T or CA treatment did not induce these behaviors. These results suggest an important role for androgens in controlling courtship in male bluegill, an effect that may depend on the sensitivity of androgen signaling as a function of spawning or parental care phase. Thus, teleost fishes, like bluegill, may provide a unique opportunity to dissect the role of sex steroids in modulating aggression and mating across distinct life-history stages.

Finally, pharmacological approaches can be used to characterize the regulation of neural circuitry underlying aggressive and reproductive behaviors via sex steroids. In teleosts, reproductive or territorial state can be expressed via a variety of signals. For example, male plainfin midshipman (*Porichthys notatus*) perform vocalizations that function for courtship and agonistic purposes[128]. Remage-Healey and Bass assessed whether different steroid hormones modulate these vocalizations by combining pharmacological manipulations with electrophysiology. Male midshipman vocalizations are controlled by a hindbrain–spinal circuit, which regulates the frequency and duration of neuronal firing in the rhythmic vocal motor system. This output directly determines the pair of muscles that modulate the fundamental frequency and duration of vocalizations. Thus, by recording from neurons in the occipital nerve roots, researchers can measure “fictive” vocalizations in restrained animals in controlled electrophysiology settings, where distinct pharmacological manipulations can be performed. The authors found that androgens, glucocorticoids, and estrogens modulate the duration of vocalizations generated by male midshipman within 15 minutes of administration. These findings suggest that different steroid hormones released during social interactions can rapidly alter courtship and agonistic behaviors.

3.2.1. A focus on pharmacological studies in A. burtoni. In the African cichlid *A. burtoni*, the use of different ER, AR, and PR agonists and antagonists, as well as aromatase inhibitors, have enhanced our understanding of the functions of estrogens, androgens, and progestins in controlling aggression and mating (Table 1). The first study to investigate the hormonal modulation of behavior in *A. burtoni* manipulated androgen levels in males using the synthetic androgen testosterone propionate. In this experiment, Fernald[92] found that testosterone propionate administration enhances body coloration and attacks towards male conspecifics, but does not alter courtship behavior.

Other pharmacology experiments in *A. burtoni* demonstrate the complex regulation of social behavior by steroid hormones. O’Connell and Hofmann[93] injected DOM or SUB males with different ER, AR, and PR agonists and antagonists and measured aggressive, mating, and submissive behaviors. Male status was confirmed visually before fish were injected with one of the pharmacological treatments. E_2 injections increased aggression in DOM and SUB males, while injections of the ER antagonist ICI182780 reduced aggression in DOM males. Surprisingly, after a slight reduction in SUB male aggression following ER antagonism, rates of aggression paralleled those of the fish given the ER agonist. This pattern was not observed in DOM males, suggesting that the modulation of DOM-typical behaviors by estrogens is dependent on social status.

Prior work also suggests roles for androgens and progestins in regulating aggressive and reproductive behaviors. In DOM male *A. burtoni*, 5α-dihydrotestosterone (DHT) administration stimulates, whereas CA reduces courtship behavior. These manipulations do not alter aggression, however. In contrast, neither DHT nor CA affects the rate of courtship or aggressive behaviors displayed by SUB males. PR manipulations also affect distinct behaviors in *A. burtoni* males. 5α-dihydroprogesterone (DHP) treatment increases courtship but not aggression in DOM males, while treatment with the PR antagonist ZK0112993 reduces courtship but not aggression. In SUB males, DHP administration does not impact aggression or courtship but reduces fleeing. Furthermore, male *A. burtoni* injected with the aromatase inhibitor FAD perform fewer aggressive behaviors compared to uninjected males, while courtship behaviors are not impacted[94]. Collectively, these results highlight the complexity of distinct sex steroid signaling systems in controlling social behaviors in *A. burtoni* in a status-specific manner.

The findings from O'Connell and Hofmann led Alward *et al.*[58] to hypothesize that androgen signaling is critical for social ascent in male *A. burtoni.* Specifically, because AR receptors alter courtship behavior in DOM, but not SUB males, yet ER manipulation alters aggression in both DOM and SUB males, both social opportunity and androgen signaling may be important in enabling a rise in rank from SUB to DOM social status. To test this hypothesis, experimentally suppressed SUB males were injected with CA before being given the opportunity to ascend in social status using an established paradigm. In this assay, a suppressed focal male is housed with a larger suppressor male and three females in a central compartment, and identical social environments containing males smaller than the focal male are located on either side of this central compartment. To provide an opportunity for social ascent, the larger suppressor male is removed during the middle of the night. When the lights come on in the morning, the suppressed male identifies this social opportunity and ascends. This approach has been used frequently and reliably induces social ascent in *A. burtoni* in a controlled experimental setup[58,129–132]. In this study, SUB males injected with vehicle ascended after removal of the suppressor male, exhibiting increased eye-bar intensity and body coloration and elevated aggression and courtship behaviors. Conversely, SUB males injected with CA showed all of the above features, but displayed deficits in courtship behavior following social ascent. Thus, these results suggest that androgens regulate courtship and support the hypothesis that androgen signaling and social opportunity combine to enable social ascent.

3.3. Gene editing in teleosts: Emerging comparative models for characterizing the neuroendocrine regulation of social rank

In recent years, gene editing methodologies been used to generate a variety of mutant teleost fishes lacking functional ARs, ERs, or PRs, which are particularly useful models for examining the functional significance of sex steroids in controlling social rank. To date, sex steroid signaling systems have been genetically modified in zebrafish, *A. burtoni*, medaka, and tilapia. In this section, we discuss relevant findings from each species and place a special emphasis on *A. burtoni,* as this species has been studied most extensively with respect to the hormonal regulation of social status.

3.3.1. Zebrafish. Zebrafish lacking functional ARs were made using two gene editing approaches: CRISPR/Cas9 (Clustered Regularly Interspersed Short Palindromic Repeats) and TALENs (Transcription Activator-Like Effector Nucleases)[100]. In this study, courtship behaviors were assessed in wild-type (WT) males, which possess two functional AR alleles, and knockout (KO) males, which possess two non-functional AR alleles. WT males performed more courtship behaviors than KO males, indicating that androgenic signaling is required for courtship in male zebrafish. Moreover, the roles of androgen and progesterone signaling in regulating aggression have been investigated by developing AR and PR mutant male zebrafish via CRISPR/Cas9[98]. AR KO fish perform fewer attacks compared to both WT and PR KO fish, whereas PR KO fish perform more attacks compared to both groups. These results suggest that ARs stimulate aggressive behavior, while PR inhibits aggression in male zebrafish. ER KO and aromatase gene knockout zebrafish have also been made[99,133–136], but these mutants have only been used for studies focused on reproductive development and have yet to be used to examine social status traits, such as male-typical aggression or courtship. These mutants have the potential to reveal novel insight into the complex roles played by distinct ERs and aromatase genes in regulating physiological processes and behaviors linked with social rank.

3.3.2. A. burtoni. Our lab used CRISPR/Cas9 gene editing to generate *A. burtoni* lacking functional ARα, ARβ, or both[26]. We found that DOM ARα mutant males perform fewer mating and territorial behaviors than DOM WT males, but exhibit other DOM-typical traits, including large testes and bright coloration. Conversely, DOM ARβ mutant males display WT-typical levels of mating and territorial behaviors, yet possess abnormally small testes and drab coloration. Interestingly, both DOM ARα and ARβ mutant males perform WT levels of a physical aggressive behavior called male attacks. Males lacking both receptors (ARαβ mutants) do not perform attacks towards males, however. Taken together, these results suggest that reproductive physiology and behaviors relevant to social status are regulated by distinct AR genes in *A. burtoni*, suggesting that non-redundant mechanisms control different traits of social rank. No ER or aromatase mutant *A. burtoni* have bene engineered, but these fish would provide an excellent opportunity to investigate the role of estrogenic signaling pathways in regulating social status in future work.

3.3.3. Medaka. ER mutant medaka have been used for studies focused on reproductive development and female mating behavior, but not male-typical aggression or courtship[137–139]. In a recent study, AR mutant medaka were used to determine the role of distinct AR genes (ARα and ARβ) on male-typical reproductive physiology, morphology, and courtship behavior[95]. In terms of results pertaining to the role of sex steroid hormones in controlling social dominance, this study revealed that ARα is necessary for tooth enlargement and a reproductive behavior that stimulates receptivity in females, whereas ARβ is required for fin morphogenesis and sexual motivation in males.

3.3.4. Nile tilapia. Nile tilapia lacking functional ERs and aromatase genes have been engineered as well[140–142], but these mutants have not been used to investigate male- or female-typical mating or aggressive behaviors, which would enhance our understanding of the hormonal basis of social dominance. ER, aromatase, and other genetic mutant tilapia may be especially useful for revealing conserved or divergent mechanisms underlying the neuroendocrine control of social status, as this group of teleosts is particularly diverse with respect to reproductive strategies and social structures across species.

4. Discussion

Social hierarchies are an essential component of group living for many animals[1,2,6,7]. Although the neuroendocrine control of behaviors associated with dominance (e.g., aggression, mating) have been studied for decades[22,143–147], we know little about the specific neural and molecular mechanisms through which sex steroids act to regulate social rank, especially in non-traditional animal models. In this review, we highlighted correlational, pharmacological, and molecular genetic studies on the regulation of social rank by sex steroids in teleost fishes. Specifically, we discussed how prior research has provided strong evidence of a relationship between circulating sex steroid levels, their mechanisms of action, and DOM-typical behaviors (e.g., aggression, mating) in males across species of teleosts. Furthermore, we highlighted recent studies that have integrated gene editing approaches to study the hormonal control of social status. Taken together, this research demonstrates the utility of using both pharmacological and state-of-the-art genetic tools to characterize how sex steroids regulate social status, both within and among teleost species.

Teleost fishes form the largest and most speciose vertebrate clade and, thus, exhibit remarkable variation in reproductive strategies, sexual systems, and social structures, among both distant and closely related species (Figure 1)[148–150]. Despite this

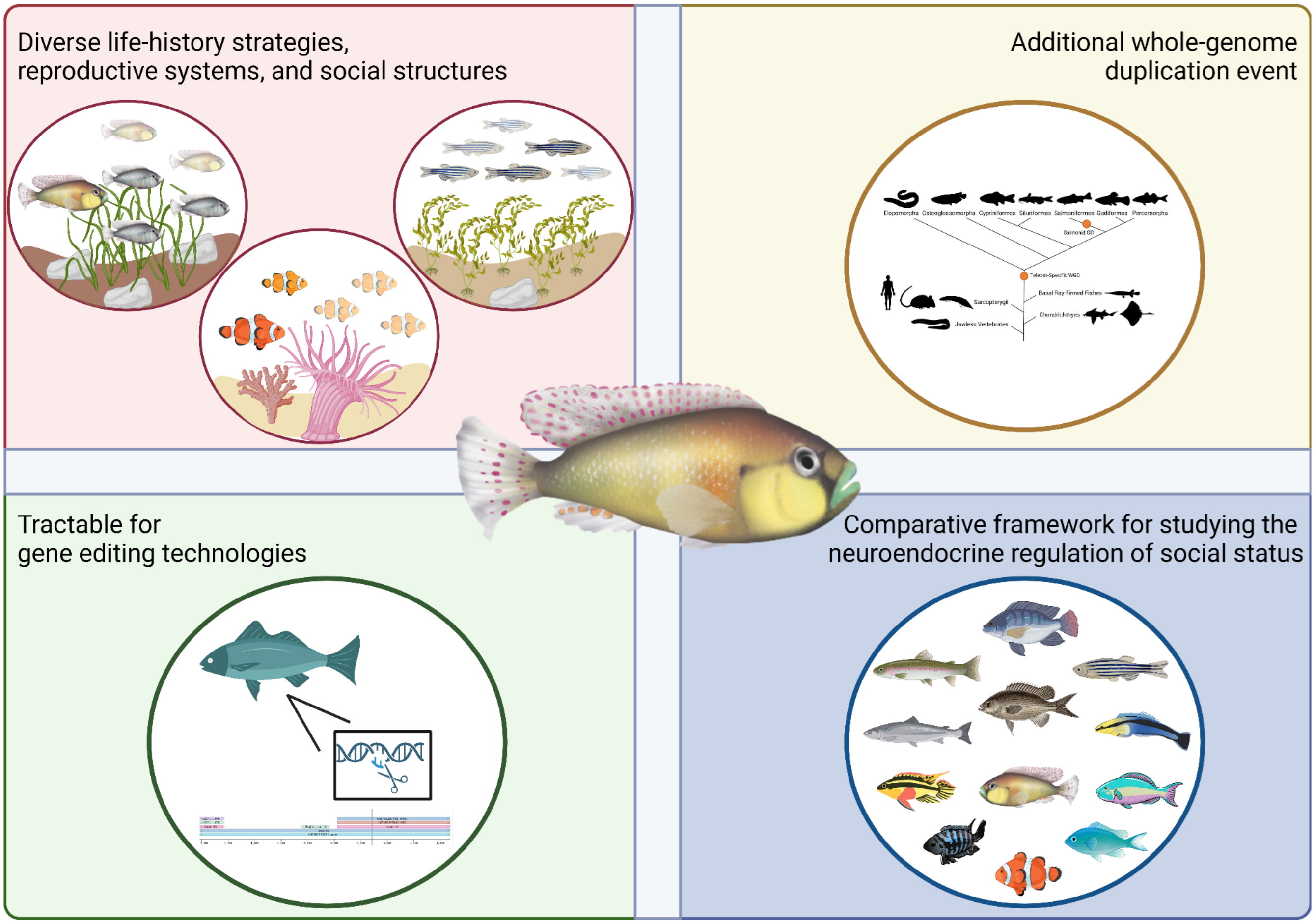

Figure 1. Summary of advantages of using teleost fishes to study the neuroendocrine regulation of social status by sex steroids. (Top left) Teleosts exhibit diverse life-history strategies, including variation in reproductive systems and social structures. For example, the African cichlid fish *Astatotilapia burtoni* (left circle) has a polygamous mating system with a male-specific social hierarchy, in which large dominant males (yellow) possess territories that are used to mate with females (gray) and actively defend their territories from small subordinate males (transparent yellow). In contrast, anemonefish (*Amphiprion* and *Premnas* sp.; center circle) have a monogamous mating system with a non-sex-specific social hierarchy, in which a large dominant female (dark orange) mates with a small male (light orange) and actively defends their territory from subordinate, non-breeding individuals (transparent light orange). Zebrafish (*Danio rerio*; right circle) have a polygamous mating system with sex-specific social hierarchies, in which large dominant males and females (blue and white) defend their territories against small subordinate individuals of the same sex (transparent blue and white). **(Top right)** Teleosts possess duplicate gene paralogs due to a teleost-specific whole-genome duplication and, in some cases, order-specific whole-genome duplication events (e.g., Salmoniformes). **(Center)** The African cichlid *A. burtoni* has been an important model organism for characterizing the role of sex steroids in modulating social status. **(Bottom left)** Teleosts are tractable model organisms for using state-of-the-art genetic tools, such as CRISPR/Cas9 gene editing. **(Bottom right)** Teleosts provide an excellent opportunity to study the evolution of the neuroendocrine control of social status by sex steroids using a comparative approach.

extraordinary diversity, the role of sex steroids in controlling social rank has been studied in surprisingly few species. Teleosts provide an excellent opportunity to elucidate the neural and molecular mechanisms that regulate social rank and to examine how these mechanisms may differ among distant or closely related species that exhibit comparable or distinct life-history strategies. For example, cichlids (family Cichlidae) show remarkable diversity in their reproductive strategies and social structures, even among closely related species. The African cichlid *A. burtoni* exhibits a polygamous mating system, in which males form a dominance hierarchy and females exclusively provide maternal care[130,151]. Conversely, the African cichlid *N. pulcher* has a monogamous mating system, in which all individuals in a population form a dominance hierarchy. In this species, social groups consist of a DOM mating pair and SUB helpers of both sexes, which provide parental care to offspring[152–154]. Some teleosts also exhibit dominance hierarchies within a sexually dynamic system, in which individuals undergo sex change during adulthood in response to changes in their social environment. The most well-studied models of the neural and hormonal control of social rank and its associated behaviors are sex-changing species of reef fish, including bluehead wrasse (*Thalassoma bifasciatum*), anemonefish (*Amphiprion* and *Premnas* sp.), and bluebanded gobies (*Lythrypnus dalli*)[155–158]. Future research that uses a comparative framework to characterize the neural and molecular mechanisms controlling social status, especially across species with diverse life-history strategies, will be an important tool for studying the evolution of social behavior and its underlying processes in teleost fishes.

Additionally, most studies on sex steroids and social status in teleosts have focused primarily on DOM individuals within social hierarchies. Thus, little is known about the neuroendocrine control of behavior in SUB individuals. While few studies have directly assessed the role of sex steroids in regulating traits associated with subordination, circulating steroid levels (especially androgens) are typically negatively associated with a SUB social rank[159–161]. There is also considerable evidence that sex steroids interact with other neuromodulators, such as neurotransmitters, to regulate the brain and behavior[162–165]. Further studies are necessary to elucidate the functions of sex steroid hormones in controlling SUB-typical behaviors and to assess whether these mechanisms differ between DOM and SUB individuals. Moreover, prior work that has examined how sex steroids regulate social rank in teleosts has used species in which males form social hierarchies. Thus, the functions of sex steroids in regulating social status in female fish, as well as potential sex differences in these underlying mechanisms, are relatively unexplored. These questions can be addressed using teleost species that exhibit female- or non-sex-specific social hierarchies. For example, zebrafish exhibit female-female dominance hierarchies within their social groups[166,167], whereas in the African cichlid *N. pulcher*, one dominance hierarchy exists among all individuals in a social group, regardless of sex[152,154,168]. Additional research is needed to assess sex differences in the regulation of social rank by sex steroids and how these mechanisms may differ among species with different social structures.

Finally, research on the control of social status by sex steroids in teleosts will benefit by using state-of-the-art genetic methodologies (Figure 1)[19,169,170]. To date, gene editing has only been utilized in a few teleost species, including zebrafish, *A. burtoni*, medaka, and Nile tilapia. The advent of this methodology in teleosts, along with the increased availability and reduced cost of whole-genome sequencing applications in recent years, will make gene editing a feasible approach for studying the neural and molecular mechanisms controlling social status across teleost species. Teleosts also represent a novel opportunity for disentangling the effects of sex steroids on social status and its associated behaviors due to a teleost-specific whole-genome duplication[19,170]. Indeed, there is emerging evidence that some steroid receptors and enzymes within the steroidogenic pathway, including ARs (ARα and ARβ) and aromatase (*cyp19a1* and c*yp19a1a*), have undergone subfunctionalization, in which both paralogs have been retained due to the complementary division of ancestral functions[45]. For example, teleosts have two androgen receptors, ARα and ARβ, which have distinct roles in regulating traits associated with a DOM social status in male *A. burtoni*. ARα mutants generated via CRISPR/Cas9 gene editing are brightly colored and have large testes, but show deficits in aggressive and mating behaviors, whereas ARβ mutants lack bright coloration and show reduced testes size, but perform normal levels of DOM-typical behaviors[26]. Moreover, because teleosts possess novel duplicate paralogs of ancestral genes, pharmacological manipulations provide little insight into the functions of specific steroid receptors and their synthetic enzymes, as this approach often involves the use of agonists or antagonists that block all subtypes of a steroid receptor or steroidogenic enzyme. Thus, the specificity of gene editing and its more widespread use in teleost fishes will enhance our understanding of how novel steroid receptors and steroidogenic enzymes, such as ARα and ARβ, control social status and will enable researchers to assess the neuroendocrine regulation of social behavior using a comparative framework.

5. Conclusions

Many species are inherently social, and dominance hierarchies are an essential component of establishing and maintaining stability in group-living animals. While prior research has characterized how sex steroids control aggressive and reproductive behaviors, relatively few studies have examined the neural and molecular mechanisms through which sex steroids regulate social status and its associated behaviors using model organisms that exhibit dominance hierarchies. Teleost fishes are excellent models for addressing these outstanding questions, as they display remarkable variation in their life-history strategies, including reproductive systems and social structures, often among closely related species. Moreover, teleosts possess novel duplicate paralogs of steroid-related genes due to a teleost-specific whole genome duplication, enabling researchers to disentangle the roles of sex steroids in regulating distinct traits associated with social rank. With the advent of gene editing technologies, such as CRISPR/Cas9, teleost fishes will be invaluable models for elucidating how sex steroids and other neuroendocrine substrates modulate traits linked with social status. More broadly, future research that implements these state-of-the-art tools in teleosts using interdisciplinary and comparative approaches will provide critical insight into how these mechanisms have evolved, and will continue to evolve, in vertebrates.

Data availability

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References

1. Fernald RD: **Cognitive skills and the evolution of social systems.** *J Exp Biol.* 2017; **220**(Pt 1): 103–113.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**
2. Sapolsky RM: **The influence of social hierarchy on primate health.** *Science.* 2005; **308**(5722): 648–653.
PubMed Abstract | **Publisher Full Text**
3. Rowell TE: **The concept of social dominance.** *Behav Biol.* 1974; **11**(2): 131–54.
PubMed Abstract | **Publisher Full Text**
4. Wingfield JC, Sapolsky RM: **Reproduction and resistance to stress: when and how.** *J Neuroendocrinol.* 2003; **15**(8): 711–724.
PubMed Abstract | **Publisher Full Text**
5. Zhou T, Sandi C, Hu H: **Advances in understanding neural mechanisms of social dominance.** *Curr Opin Neurobiol.* 2018; **49**: 99–107.
PubMed Abstract | **Publisher Full Text**
6. Milewski TM, Lee W, Champagne FA, *et al.*: **Behavioural and physiological plasticity in social hierarchies.** *Philos Trans R Soc Lond B Biol Sci.* 2022; **377**(1845): 20200443.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**
7. Tibbetts EA, Pardo-Sanchez J, Weise C: **The establishment and maintenance of dominance hierarchies.** *Philos Trans R Soc Lond B Biol Sci.* 2022; **377**(1845): 20200450.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**
8. Williamson CM, Lee W, Curley JP: **Temporal dynamics of social hierarchy formation and maintenance in male mice.** *Anim Behav.* 2016; **115**: 259–272.
Publisher Full Text
9. Van Den Berg WE, Lamballais S, Kushner SA: **Sex-Specific mechanism of social hierarchy in mice.** *Neuropsychopharmacology.* 2015; **40**(6): 1364–1372.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**
10. Anyan JJ, Seney ML, Holley A, *et al.*: **Social status and sex effects on neural morphology in Damaraland mole-rats, *Fukomys damarensis*.** *Brain Behav Evol.* 2011; **77**(4): 291–298.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**
11. Clarke FM, Faulkes CG: **Dominance and queen succession in captive colonies of the eusocial naked mole-rat, *Heterocephalus glaber***. *Proc Biol Sci.* 1997; **264**(1384): 993–1000.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**
12. Yosida S, Okanoya K: **Naked mole-rat is sensitive to social hierarchy encoded in antiphonal vocalization.** *Ethology.* 2009; **115**(9): 823–831.
Publisher Full Text
13. Barker TH, George RP, Howarth GS, *et al.*: **Assessment of housing density, space allocation and social hierarchy of laboratory rats on behavioural measures of welfare.** *PLoS One.* 2017; **12**(9): e0185135.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**
14. Fernald RD: **Cognitive skills needed for social hierarchies.** *Cold Spring Harb Symp Quant Biol.* 2014; **79**: 229–236.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**
15. Fox SF, Rose E, Myers R: **Dominance and the acquisition of superior home ranges in the lizard uta stansburiana.** *Ecology.* 1981; **62**(4): 888–893.
Publisher Full Text
16. López P, Muñoz A, Martin J: **Symmetry, male dominance and female mate preferences in the Iberian rock lizard, *Lacerta monticola*.** *Behav Ecol Sociobiol.* 2002; **52**: 342–347.
Publisher Full Text
17. Stamps JA, Krishnan VV: **Territory acquisition in lizards. IV. Obtaining high status and exclusive home ranges.** *Anim Behav.* 1998; **55**(2): 461–72.
PubMed Abstract | **Publisher Full Text**
18. Adkins-Regan E: **Hormones and animal social behavior.** (Princeton University Press), 2005.
Reference Source
19. Alward BA, Hoadley AP, Jackson LR, *et al.*: **Genetic dissection of steroid-hormone modulated social behavior: novel paralogous genes are a boon for discovery.** *Horm Behav.* 2023; **147**: 105295.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**
20. Almeida O, Gonçalves-de-Freitas E, Lopes JS, *et al.*: **Social instability promotes hormone-behavior associated patterns in a cichlid fish.** *Horm Behav.* 2014; **66**(2): 369–382.
PubMed Abstract | **Publisher Full Text**
21. Maguire SM, DeAngelis R, Dijkstra PD, *et al.*: **Social network dynamics predict hormone levels and behavior in a highly social cichlid fish.** *Horm Behav.* 2021; **132**: 104994.
PubMed Abstract | **Publisher Full Text**
22. Maruska KP, Anselmo CM, King T, *et al.*: **Endocrine and neuroendocrine regulation of social status in cichlid fishes.** *Horm Behav.* 2022; **139**: 105110.
PubMed Abstract | **Publisher Full Text**
23. Oliveira RF, Hirschenhauser K, Carneiro LA, *et al.*: **Social modulation of androgen levels in male teleost fish.** *Comp Biochem Physiol B Biochem Mol Biol.* 2002; **132**(1): 203–215.
PubMed Abstract | **Publisher Full Text**
24. Fan Z, Chang J, Liang Y, *et al.*: **Neural mechanism underlying depressive-like state associated with social status loss.** *Cell.* 2023; **186**(3): 560–576.e17.
PubMed Abstract | **Publisher Full Text**
25. Mosher LJ, Godar SC, Morissette M, *et al.*: **Steroid 5α-reductase 2 deficiency leads to reduced dominance-related and impulse-control behaviors.** *Psychoneuroendocrinology.* 2018; **91**: 95–104.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**
26. Alward BA, Laud VA, Skalnik CJ, *et al.*: **Modular genetic control of social status in a cichlid fish.** *Proc Natl Acad Sci U S A.* 2020; **117**(45): 28167–28174.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**
27. Eick GN, Thornton JW: **Evolution of steroid receptors from an estrogen-sensitive ancestral receptor.** *Mol Cell Endocrinol.* 2011; **334**(1–2): 31–38.
PubMed Abstract | **Publisher Full Text**
28. Bridgham JT, Carroll SM, Thornton JW: **Evolution of hormone-receptor complexity by molecular exploitation.** *Science.* 2006; **312**(5770): 97–101.
PubMed Abstract | **Publisher Full Text**
29. Young LJ, Crews D: **Comparative neuroendocrinology of steroid receptor gene expression and regulation: relationship to physiology and behavior.** *Trends Endocrinol Metab.* 1995; **6**(9–10): 317–323.
PubMed Abstract | **Publisher Full Text**
30. Guerriero G: **Vertebrate sex steroid receptors: evolution, ligands, and neurodistribution.** *Ann N Y Acad Sci.* Blackwell Publishing Inc., 2009; **1163**: 154–168.
PubMed Abstract | **Publisher Full Text**
31. Pfaff DW, Rubin RT, Schneider JE, *et al.*: **Hormone receptors act by multiple interacting mechanisms.** In: *Principles of Hormone/Behavior Relations*. (eds. Pfaff, D. W., Rubin, R. T., Schneider, J. E. & Head, G. A.) Elsevier, 2018; 358–364.
Publisher Full Text

32. Do Rego JL, Seong JY, Burel D, *et al.*: **Regulation of neurosteroid biosynthesis by neurotransmitters and neuropeptides.** *Front Endocrinol (Lausanne).* 2012; **3**: 4.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

33. Forlano PM, Schlinger BA, Bass AH: **Brain aromatase: new lessons from non-mammalian model systems.** *Front Neuroendocrinol.* 2006; **27**(3): 247–274.
PubMed Abstract | **Publisher Full Text**

34. Schmidt KL, Pradhan DS, Shah AH, *et al.*: **Neurosteroids, immunosteroids, and the Balkanization of endocrinology.** *Gen Comp Endocrinol.* 2008; **157**(3): 266–274.
PubMed Abstract | **Publisher Full Text**

35. Payne AH, Hales DB: **Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones.** *Endocr Rev.* 2004; **25**(6): 947–970.
PubMed Abstract | **Publisher Full Text**

36. Tokarz J, Möller G, De Angelis MH, *et al.*: **Steroids in teleost fishes: a functional point of view.** *Steroids.* 2015; **103**: 123–144.
PubMed Abstract | **Publisher Full Text**

37. Imamichi Y, Yuhki KI, Orisaka M, *et al.*: **11-ketotestosterone is a major androgen produced in human gonads.** *J Clin Endocrinol Metab.* 2016; **101**(10): 3582–3591.
PubMed Abstract | **Publisher Full Text**

38. Yazawa T, Uesaka M, Inaoka Y, *et al.*: **Cyp11b1 is induced in the murine gonad by luteinizing hormone/human chorionic gonadotropin and involved in the production of 11-ketotestosterone, a major fish androgen: conservation and evolution of the androgen metabolic pathway.** *Endocrinology.* 2008; **149**(4): 1786–1792.
PubMed Abstract | **Publisher Full Text**

39. Norris DO, Carr JA: **Vertebrate Endocrinology.** Academic Press, 2020.
Publisher Full Text

40. Thomas P, Converse A, Berg HA: **ZIP9, a novel membrane androgen receptor and zinc transporter protein.** *Gen Comp Endocrinol.* 2018; **257**: 130–136.
PubMed Abstract | **Publisher Full Text**

41. Le Ferrec E, Øvrevik J: **G-protein coupled receptors (GPCR) and environmental exposure. Consequences for cell metabolism using the β-adrenoceptors as example.** *Curr Opin Toxicol.* 2018; **8**: 14–19.
Publisher Full Text

42. Glasauer SMK, Neuhauss SCF: **Whole-genome duplication in teleost fishes and its evolutionary consequences.** *Mol Genet Genomics.* 2014; **289**(6): 1045–1060.
PubMed Abstract | **Publisher Full Text**

43. Brunet FG, Crollius HR, Paris M, *et al.*: **Gene loss and evolutionary rates following whole-genome duplication in teleost fishes.** *Mol Biol Evol.* 2006; **23**(9): 1808–1816.
PubMed Abstract | **Publisher Full Text**

44. Ohno S: **Evolution by gene duplication.** Springer, 1970.
Publisher Full Text

45. Force A, Lynch M, Pickett FB, *et al.*: **Preservation of duplicate genes by complementary, degenerative mutations.** *Genetics.* 1999; **151**(4): 1531–1545.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

46. Postlethwait J, Amores A, Cresko W, *et al.*: **Subfunction partitioning, the teleost radiation and the annotation of the human genome.** *Trends Genet.* 2004; **20**(10): 481–490.
PubMed Abstract | **Publisher Full Text**

47. Santos ME, Salzburger W: **Evolution. How cichlids diversify.** *Science.* 2012; **338**(6107): 619–620.
PubMed Abstract | **Publisher Full Text**

48. Heule C, Salzburger W, Böhne A: **Genetics of sexual development: an evolutionary playground for fish.** *Genetics.* 2014; **196**(3): 579–591.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

49. Lorin T, Salzburger W, Böhne A: **Evolutionary fate of the androgen receptor-Signaling pathway in ray-finned fishes with a special focus on cichlids.** *G3 (Bethesda).* 2015; **5**(11): 2275–2283.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

50. Brawand D, Wagner CE, Li YI, *et al.*: **The genomic substrate for adaptive radiation in African cichlid fish.** *Nature.* 2014; **513**(7518): 375–381.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

51. Munley KM, Hoadley AP, Alward BA, *et al.*: **A phylogenetics-based nomenclature system for steroid receptors in teleost fishes.** *Gen Comp Endocrinol.* 2024; **347**: 114436.
PubMed Abstract | **Publisher Full Text**

52. Krause J, Ruxton GD: **Living in Groups.** Oxford University Press, 2002.
Publisher Full Text

53. Taborsky M, Wong MY: **Sociality in fishes.** In: *Comparative Social Evolution.* (eds. Rubenstein, D. I. & Abbot, P.) Cambridge University Press, 2017; 354–389.
Publisher Full Text

54. Creel S: **Social dominance and stress hormones.** *Trends Ecol Evol.* 2001; **16**(9): 491–497.
Publisher Full Text

55. Gilmour KM, Dibattista JD, Thomas JB: **Physiological causes and consequences of social status in salmonid fish.** *Integr Comp Biol.* 2005; **45**(2): 263–273.
PubMed Abstract | **Publisher Full Text**

56. Knight EL, Sarkar A, Prasad S, *et al.*: **Beyond the challenge hypothesis: The emergence of the dual-hormone hypothesis and recommendations for future research.** *Horm Behav.* 2020; **123**: 104657.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

57. Sherman GD, Mehta PH: **Stress, cortisol, and social hierarchy.** *Curr Opin Psychol.* 2020; **33**: 227–232.
PubMed Abstract | **Publisher Full Text**

58. Alward BA, Hilliard AT, York RA, *et al.*: **Hormonal regulation of social ascent and temporal patterns of behavior in an African cichlid.** *Horm Behav.* 2019; **107**: 83–95.
PubMed Abstract | **Publisher Full Text**

59. Maruska KP, Fernald RD: **Behavioral and physiological plasticity: rapid changes during social ascent in an African cichlid fish.** *Horm Behav.* 2010; **58**(2): 230–240.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

60. Maruska KP, Zhang A, Neboori A, *et al.*: **Social opportunity causes rapid transcriptional changes in the Social Behaviour Network of the brain in an African cichlid fish.** *J Neuroendocrinol.* 2013; **25**(2): 145–157.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

61. O'Connell LA, Ding JH, Hofmann HA, *et al.*: **Sex differences and similarities in the neuroendocrine regulation of social behavior in an African cichlid fish.** *Horm Behav.* 2013; **64**(3): 468–476.
PubMed Abstract | **Publisher Full Text**

62. Parikh VN, Clement TS, Fernald RD: **Androgen level and male social status in the African cichlid, *Astatotilapia burtoni*.** *Behav Brain Res.* 2006; **166**(2): 291–295.
PubMed Abstract | **Publisher Full Text**

63. Golan M, Levavi-Sivan B: **Social dominance in tilapia is associated with gonadotroph hyperplasia.** *Gen Comp Endocrinol.* 2013; **192**: 126–135.
PubMed Abstract | **Publisher Full Text**

64. Pfennig F, Kurth T, Meissner S, *et al.*: **The social status of the male Nile tilapia (*Oreochromis niloticus*) influences testis structure and gene expression.** *Reproduction.* 2012; **143**(1): 71–84.
PubMed Abstract | **Publisher Full Text**

65. Aubin-Horth N, Desjardins JK, Martei YM, *et al.*: **Masculinized dominant females in a cooperatively breeding species.** *Mol Ecol.* 2007; **16**(7): 1349–1358.
PubMed Abstract | **Publisher Full Text**

66. Desjardins JK, Stiver KA, Fitzpatrick JL, *et al.*: **Sex and status in a cooperative breeding fish: behavior and androgens.** *Behav Ecol Sociobiol.* 2008; **62**(5): 785–794.
Publisher Full Text

67. Taves MD, Desjardins JK, Mishra S, *et al.*: **Androgens and dominance: Sex-specific patterns in a highly social fish (*Neolamprologus pulcher*).** *Gen Comp Endocrinol.* 2009; **161**(2): 202–207.
PubMed Abstract | **Publisher Full Text**

68. Morandini L, Honji RM, Ramallo MR, *et al.*: **The interrenal gland in males of the cichlid fish *Cichlasoma dimerus*: relationship with stress and the establishment of social hierarchies.** *Gen Comp Endocrinol.* 2014; **195**: 88–98.
PubMed Abstract | **Publisher Full Text**

69. Ramallo MR, Birba A, Honji RM, *et al.*: **A multidisciplinary study on social status and the relationship between inter-individual variation in hormone levels and agonistic behavior in a Neotropical cichlid fish.** *Horm Behav.* 2015; **69**: 139–151.
PubMed Abstract | **Publisher Full Text**

70. Dijkstra PD, Hekman R, Schulz RW, *et al.*: **Social stimulation, nuptial colouration, androgens and immunocompetence in a sexual dimorphic cichlid fish.** *Behav Ecol Sociobiol.* 2007; **61**(4): 599–609.
Publisher Full Text

71. Cardwell JR, Sorensen PW, Van der Kraak GJ, *et al.*: **Effect of dominance status on sex hormone levels in laboratory and wild-spawning male trout.** *Gen Comp Endocrinol.* 1996; **101**(3): 333–341.
PubMed Abstract | **Publisher Full Text**

72. Liley NR, Kroon FJ: **Male dominance, plasma hormone concentrations, and availability of milt in male rainbow trout (*Oncorhynchus mykiss*).** *Canadian Journal of Zoology/Revue Canadienne de Zoologie.* 1995; **73**(5): 826–836.
Publisher Full Text

73. Elofsson UO, Mayer I, Damsgård B, *et al.*: **Intermale competition in sexually mature arctic charr: effects on brain monoamines, endocrine stress responses, sex hormone levels, and behavior.** *Gen Comp Endocrinol.* 2000; **118**(3): 450–460.
PubMed Abstract | **Publisher Full Text**

74. Cardwell JR, Liley NR: **Androgen control of social status in males of a wild population of stoplight parrotfish, *Sparisoma viride* (Scaridae).** *Horm Behav.* 1991; **25**(1): 1–18.
PubMed Abstract | **Publisher Full Text**

75. Pankhurst NW, Barnett CW: **Relationship of population density, territorial interaction and plasma levels of gonadal steroids in spawning male demoiselles *Chromis dispilus* (Pisces: Pomacentridae).** *Gen Comp Endocrinol.* 1993; **90**(2): 168–176.
PubMed Abstract | **Publisher Full Text**

76. Stiver KA, Harris RM, Townsend JP, *et al.*: **Neural gene expression profiles and androgen levels underlie alternative reproductive tactics in the ocellated wrasse, *Symphodus ocellatus*.** *Ethology.* 2015; **121**(2): 152–167.
Publisher Full Text

77. Kagawa N, Hirose S, Fujimoto K, *et al.*: **Social rank-dependent expression of**

gonadotropin-releasing hormones and kisspeptin in the medaka brain. *Gen Comp Endocrinol.* 2017; **249**: 48–54.
PubMed Abstract | **Publisher Full Text**

78. Filby AL, Paull GC, Bartlett EJ, *et al.*: **Physiological and health consequences of social status in zebrafish (*Danio rerio*).** *Physiol Behav.* 2010; **101**(5): 576–587.
PubMed Abstract | **Publisher Full Text**

79. Huffman LS, Mitchell MM, O'Connell LA, *et al.*: **Rising StARs: behavioral, hormonal, and molecular responses to social challenge and opportunity.** *Horm Behav.* 2012; **61**(4): 631–641.
PubMed Abstract | **Publisher Full Text**

80. Maruska KP: **Social transitions cause rapid behavioral and neuroendocrine changes.** *Integr Comp Biol.* 2015; **55**(2): 294–306.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

81. Scaia MF, Morandini L, Noguera CA, *et al.*: **Fighting cichlids: dynamic of intrasexual aggression in dyadic agonistic encounters.** *Behav Process.* 2018; **147**: 61–69.
PubMed Abstract | **Publisher Full Text**

82. Dzieweczynski TL, Eklund AC, Rowland WJ: **Male 11-ketotestosterone levels change as a result of being watched in Siamese fighting fish, *Betta splendens*.** *Gen Comp Endocrinol.* 2006; **147**(2): 184–189.
PubMed Abstract | **Publisher Full Text**

83. Ramos A, Alex D, Cardoso SD, *et al.*: **Androgens and corticosteroids increase in response to mirror images and interacting conspecifics in males of the Siamese fighting fish *Betta splendens*.** *Horm Behav.* 2021; **132**: 104991.
PubMed Abstract | **Publisher Full Text**

84. Oliveira RF, Almada VC, Gonçalves EJ, *et al.*: **Androgen levels and social interactions in breeding males of the peacock blenny.** *J Fish Biol.* 2001; **58**(4): 897–908.
Publisher Full Text

85. Ros AFH, Oliveira RF: **Androgens and immune function in male alternative reproductive morphotypes of the peacock blenny *Salaria pavo*.** *Ethology.* 2009; **115**(6): 555–565.
Publisher Full Text

86. Hannes RP: **Blood and whole-body androgen levels of male swordtails correlated with aggression measures in a standard-opponent test.** *Aggress Behav.* 1986; **12**(4): 249–254.
Publisher Full Text

87. Salek SJ, Sullivan CV, Godwin J: **Courtship behavior of male white perch, *Morone americana*: evidence for control by androgens.** *Comp Biochem Physiol A Mol Integr Physiol.* 2001; **130**(4): 731–740.
PubMed Abstract | **Publisher Full Text**

88. Remage-Healey L, Bass AH: **Rapid elevations in both steroid hormones and vocal signaling during playback challenge: a field experiment in Gulf toadfish.** *Horm Behav.* 2005; **47**(3): 297–305.
PubMed Abstract | **Publisher Full Text**

89. Brantley RK, Wingfield JC, Bass AH: **Sex steroid levels in *Porichthys notatus*, a fish with alternative reproductive tactics, and a review of the hormonal bases for male dimorphism among teleost fishes.** *Horm Behav.* 1993; **27**(3): 332–347.
PubMed Abstract | **Publisher Full Text**

90. Borg B, Mayer I: **Androgens and behaviour in the three-spined stickleback.** *Behaviour.* 1995; **132**: 1025–1035.
Publisher Full Text

91. Páll MK, Mayer I, Borg B: **Androgen and behavior in the male three-spined stickleback, *Gasterosteus aculeatus* I.-- Changes in 11-ketotestosterone levels during the nesting cycle.** *Horm Behav.* 2002; **41**(4): 377–383.
PubMed Abstract | **Publisher Full Text**

92. Fernald RD: **The effect of testosterone on the behavior and coloration of adult male cichlid fish (*Haplochromis burtoni*, Günther).** *Horm Res.* 1976; **7**(3): 172–178.
PubMed Abstract

93. O'Connell LA, Hofmann HA: **Social status predicts how sex steroid receptors regulate complex behavior across levels of biological organization.** *Endocrinology.* 2012; **153**(3): 1341–1351.
PubMed Abstract | **Publisher Full Text**

94. Huffman LS, O'Connell LA, Hofmann HA: **Aromatase regulates aggression in the African cichlid fish *Astatotilapia burtoni*.** *Physiol Behav.* 2013; **112–113**: 77–83.
PubMed Abstract | **Publisher Full Text**

95. Ogino Y, Ansai S, Watanabe E, *et al.*: **Evolutionary differentiation of androgen receptor is responsible for sexual characteristic development in a teleost fish.** *Nat Commun.* 2023; **14**(1): 1428.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

96. Balch GC, Mackenzie CA, Metcalfe CD: **Alterations to gonadal development and reproductive success in Japanese medaka (*Oryzias latipes*) exposed to 17alpha-ethinylestradiol.** *Environ Toxicol Chem.* 2004; **23**(3): 782–791.
PubMed Abstract | **Publisher Full Text**

97. Oshima Y, Kang IJ, Kobayashi M, *et al.*: **Suppression of sexual behavior in male Japanese medaka (*Oryzias latipes*) exposed to 17beta-estradiol.** *Chemosphere.* 2003; **50**(3): 429–436.
PubMed Abstract | **Publisher Full Text**

98. Carver JJ, Carrell SC, Chilton MW, *et al.*: **Nuclear androgen and progestin receptors inversely affect aggression and social dominance in male zebrafish (*Danio rerio*).** *Horm Behav.* 2021; **134**: 105012.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

99. Lu H, Cui Y, Jiang L, *et al.*: **Functional analysis of nuclear estrogen receptors in zebrafish reproduction by genome editing approach.** *Endocrinology.* 2017; **158**(7): 2292–2308.
PubMed Abstract | **Publisher Full Text**

100. Yong L, Thet Z, Zhu Y: **Genetic editing of the androgen receptor contributes to impaired male courtship behavior in zebrafish.** *J Exp Biol.* 2017; **220**(Pt 17): 3017–3021.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

101. Fenske L, Concato AC, Vanin AP, *et al.*: **17-α-Ethinylestradiol modulates endocrine and behavioral responses to stress in zebrafish.** *Environ Sci Pollut Res Int.* 2020; **27**(23): 29341–29351.
PubMed Abstract | **Publisher Full Text**

102. Maruska KP, Soares MC, Lima-Maximino M, *et al.*: **Social plasticity in the fish brain: Neuroscientific and ethological aspects.** *Brain Res.* 2019; **1711**: 156–172.
PubMed Abstract | **Publisher Full Text**

103. Moore IT, Hernandez J, Goymann W: **Who rises to the challenge? Testing the Challenge Hypothesis in fish, amphibians, reptiles, and mammals.** *Horm Behav.* 2020; **123**: 104537.
PubMed Abstract | **Publisher Full Text**

104. Oliveira RF: **Social behavior in context: Hormonal modulation of behavioral plasticity and social competence.** *Integr Comp Biol.* 2009; **49**(4): 423–440.
PubMed Abstract | **Publisher Full Text**

105. Desjardins JK, Hazelden MR, Van der Kraak GJ, *et al.*: **Male and female cooperatively breeding fish provide support for the "Challenge Hypothesis".** *Behav Ecol.* 2006; **17**(2): 149–154.
Publisher Full Text

106. Oliveira RF, Silva A, Canário AVM: **Why do winners keep winning? Androgen mediation of winner but not loser effects in cichlid fish.** *Proc Biol Sci.* 2009; **276**(1665): 2249–2256.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

107. Félix AS, Cardoso SD, Roleira A, *et al.*: **Forebrain transcriptional response to transient changes in circulating androgens in a cichlid fish.** *G3 (Bethesda).* 2020; **10**(6): 1971–1982.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

108. Hirschenhauser K, Taborsky M, Oliveira T, *et al.*: **A test of the 'challenge hypothesis' in cichlid fish: simulated partner and territory intruder experiments.** *Anim Behav.* 2004; **68**(4): 741–750.
Publisher Full Text

109. Hirschenhauser K, Oliveira RF: **Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis.** *Anim Behav.* 2006; **71**(2): 265–277.
Publisher Full Text

110. Dijkstra PD, Schaafsma SM, Hofmann HA, *et al.*: **'Winner effect' without winning: unresolved social conflicts increase the probability of winning a subsequent contest in a cichlid fish.** *Physiol Behav.* 2012; **105**(2): 489–492.
PubMed Abstract | **Publisher Full Text**

111. Oliveira RF, Lopes M, Carneiro LA, *et al.*: **Watching fights raises fish hormone levels.** *Nature.* 2001; **409**(6819): 475.
PubMed Abstract | **Publisher Full Text**

112. Antunes RA, Oliveira RF: **Hormonal anticipation of territorial challenges in cichlid fish.** *Proc Natl Acad Sci U S A.* 2009; **106**(37): 15985–15989.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

113. Roleira A, Oliveira GA, Lopes JS, *et al.*: **Audience effects in territorial defense of male cichlid fish are associated with differential patterns of activation of the brain Social Decision-Making Network.** *Front Behav Neurosci.* 2017; **11**: 105.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

114. Aires RF, Oliveira GA, Oliveira TF, *et al.*: **Dear enemies elicit lower androgen responses to territorial challenges than unfamiliar intruders in a cichlid fish.** *PLoS One.* 2015; **10**(9): e0137705.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

115. Maruska KP, Fernald RD: **Steroid receptor expression in the fish inner ear varies with sex, social status, and reproductive state.** *BMC Neurosci.* 2010; **11**: 58.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

116. Scaia MF, Morandini L, Noguera C, *et al.*: **Can estrogens be considered as key elements of the challenge hypothesis? The case of intrasexual aggression in a cichlid fish.** *Physiol Behav.* 2018; **194**: 481–490.
PubMed Abstract

117. Pradhan A, Olsson PE: **Zebrafish sexual behavior: role of sex steroid hormones and prostaglandins.** *Behav Brain Funct.* 2015; **11**: 23.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

118. Segner H, Casanova-Nakayama A, Kase R, *et al.*: **Impact of environmental estrogens on Yfish considering the diversity of estrogen signaling.** *Gen Comp Endocrinol.* 2013; **191**: 190–201.
PubMed Abstract | **Publisher Full Text**

119. Teng C, Goodwin B, Shockley K, *et al.*: **Bisphenol a affects androgen receptor function via multiple mechanisms.** *Chem Biol Interact.* 2013; **203**(3): 556–564.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

120. Li X, Guo JY, Li X, *et al.*: **Behavioural effect of low-dose BPA on male zebrafish: tuning of male mating competition and female mating preference during courtship process.** *Chemosphere.* 2017; **169**: 40–52.
PubMed Abstract | **Publisher Full Text**

121. Lee PY, Lin CY, Chen TH: **Environmentally relevant exposure of 17α-ethinylestradiol impairs spawning and reproductive behavior in the brackish medaka *Oryzias melastigma*.** *Mar Pollut Bull.* 2014; **85**(2): 338–343.
PubMed Abstract | **Publisher Full Text**

122. Salierno JD, Kane AS: **17α-ethinylestradiol alters reproductive behaviors, circulating hormones, and sexual morphology in male fathead minnows (*Pimephales promelas*).** *Environ Toxicol Chem.* 2009; **28**(5): 953–961.
PubMed Abstract | **Publisher Full Text**

123. Belanger RM, Pachkowski MD, Stacey NE: **Methyltestosterone-induced changes in electro-olfactogram responses and courtship behaviors of cyprinids.** *Chem Senses.* 2010; **35**(1): 65–74.
PubMed Abstract | **Publisher Full Text**

124. van Breukelen NA: **Androgen receptor antagonist impairs courtship but not aggressive behavior in the monogamous cichlid, *Amatitlania nigrofasciata*.** *Horm Behav.* 2013; **63**(3): 527–532.
PubMed Abstract | **Publisher Full Text**

125. Jalabert C, Quintana L, Pessina P, *et al.*: **Extra-gonadal steroids modulate non-breeding territorial aggression in weakly electric fish.** *Horm Behav.* 2015; **72**: 60–67.
PubMed Abstract | **Publisher Full Text**

126. Kindler PM, Bahr JM, Philipp DP: **The effects of exogenous 11-ketotestosterone, testosterone, and cyproterone acetate on prespawning and parental care behaviors of male bluegill.** *Horm Behav.* 1991; **25**(3): 410–423.
PubMed Abstract | **Publisher Full Text**

127. Sar M, Stumpf WE: **Effects of progesterone or cyproterone acetate on androgen uptake in the brain, pituitary and peripheral tissues.** *Proc Soc Exp Biol Med.* 1973; **144**(1): 26–29.
PubMed Abstract | **Publisher Full Text**

128. Remage-Healey L, Bass AH: **Rapid, Hierarchical Modulation of Vocal Patterning by Steroid Hormones.** *J Neurosci.* 2004; **24**(26): 5892–5900.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

129. Burmeister SS, Jarvis ED, Fernald RD: **Rapid behavioral and genomic responses to social opportunity.** *PLoS Biol.* 2005; **3**(11): e363.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

130. Maruska KP, Fernald RD: ***Astatotilapia burtoni*: A model system for analyzing the neurobiology of behavior.** *ACS Chem Neurosci.* 2018; **9**(8): 1951–1962.
PubMed Abstract | **Publisher Full Text**

131. Maruska KP, Fernald RD: **Social regulation of male reproductive plasticity in an african cichlid fish.** *Integr Comp Biol.* 2013; **53**(6): 938–950.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

132. Fernald RD, Maruska KP: **Social information changes the brain.** *Proc Natl Acad Sci U S A.* 2012; **109 Suppl 2**(Suppl 2): 17194–17199.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

133. Yin Y, Tang H, Liu Y, *et al.*: **Targeted disruption of aromatase reveals dual functions of *cyp19a1a* during sex differentiation in zebrafish.** *Endocrinology.* 2017; **158**(9): 3030–3041.
PubMed Abstract | **Publisher Full Text**

134. Lau ESW, Zhang Z, Qin M, *et al.*: **Knockout of zebrafish ovarian aromatase gene (cyp19a1a) by TALEN and CRISPR/Cas9 leads to All-male offspring due to failed ovarian differentiation.** *Sci Rep.* 2016; **6**: 37357.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

135. Tang H, Chen Y, Liu Y, *et al.*: **New insights into the role of estrogens in male fertility based on findings in aromatase-deficient zebrafish.** *Endocrinology.* 2017; **158**(9): 3042–3054.
PubMed Abstract | **Publisher Full Text**

136. Shaw K, Therrien M, Lu C, *et al.*: **Mutation of brain aromatase disrupts spawning behavior and reproductive health in female zebrafish.** *Front Endocrinol (Lausanne).* 2023; **14**: 1225199.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

137. Tohyama S, Ogino Y, Lange A, *et al.*: **Establishment of estrogen receptor 1 (ESR1)-knockout medaka: ESR1 is dispensable for sexual development and reproduction in medaka, *Oryzias latipes*.** *Dev Growth Differ.* 2017; **59**(6): 552–561.
PubMed Abstract | **Publisher Full Text**

138. Kayo D, Zempo B, Tomihara S, *et al.*: **Gene knockout analysis reveals essentiality of estrogen receptor β1 (Esr2a) for female reproduction in medaka.** *Sci Rep.* 2019; **9**(1): 8868.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

139. Nishiike Y, Miyazoe D, Togawa R, *et al.*: **Estrogen receptor 2b is the major determinant of sex-typical mating behavior and sexual preference in medaka.** *Curr Biol.* 2021; **31**(8): 1699–1710.e6.
PubMed Abstract | **Publisher Full Text**

140. Yang L, Zhang X, Liu S, *et al.*: **Cyp17a1 is required for female sex determination and male fertility by regulating sex steroid biosynthesis in fish.** *Endocrinology.* 2021; **162**(12): bqab205.
PubMed Abstract | **Publisher Full Text**

141. Yan L, Feng H, Wang F, *et al.*: **Establishment of three estrogen receptors (*esr1, esr2a, esr2b*) knockout lines for functional study in Nile tilapia.** *J Steroid Biochem Mol Biol.* 2019; **191**: 105379.
PubMed Abstract | **Publisher Full Text**

142. Zhang X, Li M, Ma H, *et al.*: **Mutation of *foxl2* or *cyp19a1a* results in female to male sex reversal in XX nile tilapia.** *Endocrinology.* 2017; **158**(8): 2634–2647.
PubMed Abstract | **Publisher Full Text**

143. Quintana L, Jalabert C, Fokidis HB, *et al.*: **Neuroendocrine mechanisms underlying non-breeding aggression: common strategies between birds and fish.** *Front Neural Circuits.* 2021; **15**: 716605.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

144. Fuxjager MJ, Ryder TB, Moody NM, *et al.*: **Systems biology as a framework to understand the physiological and endocrine bases of behavior and its evolution—From concepts to a case study in birds.** *Horm Behav.* 2023; **151**: 105340.
PubMed Abstract | **Publisher Full Text**

145. Quintana L, Jalabert C, Fokidis HB, *et al.*: **Neuroendocrine mechanisms underlying non-breeding aggression: common strategies between birds and fish.** *Front Neural Circuits.* 2021; **15**: 716605.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

146. Munley KM, Rendon NM, Demas GE: **Neural androgen synthesis and aggression: Insights from a seasonally breeding rodent.** *Front Endocrinol (Lausanne).* 2018; **9**: 136.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

147. Nelson RJ, Trainor BC: **Neural mechanisms of aggression.** *Nat Rev Neurosci.* 2007; **8**(7): 536–546.
PubMed Abstract | **Publisher Full Text**

148. Nelson JS: **Fishes of the world.** (John Wiley and Sons, Hoboken, 2006), 2006.
Reference Source

149. Desjardins JK, Fernald RD: **Fish sex: why so diverse?** *Curr Opin Neurobiol.* 2009; **19**(6): 648–653.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

150. Lévêque C, Oberdorff T, Paugy D, *et al.*: **Global diversity of fish (Pisces) in freshwater.** In: *Freshwater Animal Diversity Assessment.* (eds. Balian, E. V., Lévêque, C., Segers, H. & Martens, K.). (Springer, Dordrecth), 2007; 545–567.
Publisher Full Text

151. Fernald RD, Hirata NR: **Field study of *Haplochromis burtoni*: habitats and co-habitant.** *Environ Biol Fish.* 1977; **2**: 299–308.
Publisher Full Text

152. Taborsky M: **Broodcare helpers in the cichlid fish *Lamprologus brichardi*: their costs and benefits.** *Anim Behav.* 1984; **32**(4): 1236–1252.
Publisher Full Text

153. Taborsky M: **Cichlid fishes: a model for the integrative study of social behavior.** In: *Cooperative Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior.* (eds. Koenig, W. D. & Dickinson, J. L.), (Cambridge University Press, Cambridge), 2016; 272–293.
Publisher Full Text

154. Taborsky M, Limberger D: **Helpers in fish.** *Behav Ecol Sociobiol.* 1981; **8**: 143–145.
Publisher Full Text

155. Perry AN, Grober MS: **A model for social control of sex change: interactions of behavior, neuropeptides, glucocorticoids, and sex steroids.** *Horm Behav.* 2003; **43**(1): 31–38.
PubMed Abstract | **Publisher Full Text**

156. Godwin J: **Sex change in reef fishes: behavior and physiology.** In: *Encyclopedia of Animal Behavior.* (ed. Choe, J. C), 2019; 364–372.
Publisher Full Text

157. Gemmell NJ, Todd EV, Goikoetxea A, *et al.*: **Natural sex change in fish.** In: *Curr Top Dev Biol.* (Academic Press Inc), 2019; **134**: 71–117.
PubMed Abstract | **Publisher Full Text**

158. Smiley KO, Munley KM, Aghi K, *et al.*: **Sex diversity in the 21st century: concepts, frameworks, and approaches for the future of neuroendocrinology.** *Horm Behav.* 2024; **157**: 105445.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

159. Fuxjager MJ, Forbes-Lorman RM, Coss DJ, *et al.*: **Winning territorial disputes selectively enhances androgen sensitivity in neural pathways related to motivation and social aggression.** *Proc Natl Acad Sci U S A.* 2010; **107**(27): 12393–12398.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

160. Wacker DW, Khalaj S, Jones LJ, *et al.*: **Dehydroepiandrosterone heightens aggression and increases androgen receptor and aromatase mRNA expression in the brain of a male songbird.** *J Neuroendocrinol.* 2016; **28**(12): n/a.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

161. Ketterson ED, Nolan V Jr, Wolf L, *et al.*: **Testosterone and avian life histories: effects of experimentally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco (Junco hyemalis).** *Am Nat.* 1992; **140**(6): 980–999.
Publisher Full Text

162. Hull EM, Lorrain DS, Du J, *et al.*: **Hormone-neurotransmitter interactions in the control of sexual behavior.** *Behav Brain Res.* 1999; **105**(1): 105–16.
PubMed Abstract | **Publisher Full Text**

163. Fink G, Sumner B, Rosie R, *et al.*: **Androgen actions on central serotonin neurotransmission: relevance for mood, mental state and memory.** *Behav Brain Res.* 1999; **105**(1): 53–68.
PubMed Abstract | **Publisher Full Text**

164. Teles MC, Almeida O, Lopes JS, *et al.*: **Social interactions elicit rapid shifts in functional connectivity in the social decision-making network of zebrafish.** *Proc Biol Sci.* 2015; **282**(1816): 20151099.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

165. McCarthy MM, Pfaus JG: **Steroid modulation of neurotransmitter function to alter female reproductive behavior.** *Trends Endocrinol Metab.* 1996; **7**(9): P327–P333.
PubMed Abstract | **Publisher Full Text**

166. Paull GC, Filby AL, Giddins HG, *et al.*: **Dominance hierarchies in zebrafish (*Danio rerio*) and their relationship with reproductive success.** *Zebrafish.* 2010; **7**(1): 109–117.
PubMed Abstract | **Publisher Full Text**

167. Stewart AM, Braubach O, Spitsbergen J, *et al.*: **Zebrafish models for translational neuroscience research: from tank to bedside.** *Trends Neurosci.* 2014; **37**(5): 264–278.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

168. Bruintjes R, Taborsky M: **Helpers in a cooperative breeder pay a high price to stay: effects of demand, helper size and sex.** *Anim Behav.* 2008; **75**(6): 1843–1850.
Publisher Full Text

169. Juntti S: **The future of gene-guided neuroscience research in non-traditional model organisms.** *Brain Behav Evol.* 2019; **93**(2–3): 108–121.
PubMed Abstract | **Publisher Full Text**

170. Jackson LR, Lopez MS, Alward BA: **Breaking through the bottleneck: krogh's principle in behavioral neuroendocrinology and the potential of gene editing.** *Integr Comp Biol.* 2023; **63**(2): 428–443.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Open Peer Review

Current Peer Review Status: ✓ ✓

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✓

Malgorzata Kotula-Balak
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The review by Munley deals with the sophisticated problem which is sex steroid control of social status in teleost fishes. The article is well-written with information on current pharmacological and molecular approaches. The summary illustration is helpful. Only some minor considerations are suggested.
1. It should be clearly stated that the primary source of sex hormones is gonad.
2. Nongenomic action is currently studied in detail mechanism of sex steroid action (ZIP9, GPER receptors etc.) with special emphasis on endocrine disrupting chemicals that are present too in an aguatic environment.

Is the topic of the review discussed comprehensively in the context of the current literature?
Partly

Are all factual statements correct and adequately supported by citations?
Yes

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Endocrinology

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard.

Author Response 28 Feb 2024

Beau Alward

Reviewer #2 (Malgorzata Kotula-Balak) The review by Munley deals with the sophisticated problem which is sex steroid control of social status in teleost fishes. The article is well-written with information on current pharmacological and molecular approaches. The summary illustration is helpful. Only some minor considerations are suggested.

Author Response: Thank you for this positive assessment of our work. We appreciate your comments and suggestions, which we address point-by-point below.

1. It should be clearly stated that the primary source of sex hormones is gonad.

Author Response: We have added this information to Section 2.1 of our manuscript (page 4, lines 131-133).

1. Nongenomic action is currently studied in detail mechanism of sex steroid action (ZIP9, GPER receptors etc.) with special emphasis on endocrine disrupting chemicals that are present too in an aquatic environment.

Author Response: Thank you for bringing this literature to our attention. We have added citations for this work to Section 2.1 (pages 4-5, lines 144-148).

Competing Interests: No competing interests were disclosed.

Reviewer Report 12 October 2023

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✓ **Elizabeth Adkins-Regan**
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This is an informative review of an interesting topic, the role of sex steroids and their receptors in social status in teleost fishes. As the review points out early on, it is key to distinguish between social behavior *per se* (aggression, mating, etc.) and social status and its determination. Status is the product of interactions with others and is dynamic and plastic. It is not a fixed property of an individual, but depends on the behavior of others and relationships with them. A major point of the review is the value of a comparative approach to the topic through the study of a diverse array of species, and in particular the value of studying teleost fishes, the most speciose groups of vertebrates. As the review emphasizes, teleosts show a wide variety of social systems. The sensitivity of their dominance relationships to social context and recent history show all the hallmarks of learning and cognition seen in birds and mammals, such as winner effects, bystander effects, audience effects, and dear enemy effects. Also fascinating is that teleosts underwent a whole genome duplication in their ancestry that makes them excellent candidates for gene manipulation approaches such as CRISPR/Cas9. The review will be valuable for a broad community of researchers interested in hormonal and neural mechanisms of social behavior.

There were a couple of puzzling omissions in the otherwise comprehensive coverage of the topic. One concerns 11-ketotestosterone. The authors should try to summarize whether, and if so, how, the role of 11-ketotestosterone differs from that of testosterone rather than leaving it up to the reader to try to figure this out from the table and text. Also, do teleosts have 11-ketotestosterone because of an enzyme gene duplication? Could its production conceivably be knocked out separately from that of other androgens? Also, at the end of section 3.1, where the role of estrogens in males is discussed, the authors should say something about estrogens and female status.

Also puzzling was the conclusion in the last sentence of section 3.2.1 (“and that androgen signaling and social opportunity combine to enable social ascent”). Doesn’t the result of the CA experiment argue against a role for androgen signaling in ascent?

In Figure 1, the top right box shows a second gene duplication in the Salmoniformes. This was not mentioned in the figure caption. If it is discussed in the text, the authors could state where (which section) in the caption.

There are quite a few places where there is a typo or a word is missing. For example, there are three typos toward the end of the first paragraph of section 2.1, and a word missing in the first sentence of section 3.3. Without line numbers, it is difficult to enumerate all the rest, but a careful reading should reveal them for the authors to fix.

Is the topic of the review discussed comprehensively in the context of the current literature?

Yes

Are all factual statements correct and adequately supported by citations?

Yes

Is the review written in accessible language?

Yes

Are the conclusions drawn appropriate in the context of the current research literature?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Comparative behavioral neuroendocrinology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 28 Feb 2024

Beau Alward

Comments from Reviewers: ***Reviewer #1 (Elizabeth Adkins-Regan)***

This is an informative review of an interesting topic, the role of sex steroids and their receptors in social status in teleost fishes. As the review points out early on, it is key to distinguish between social behavior per se (aggression, mating, etc.) and social status and its determination. Status is the product of interactions with others and is dynamic and plastic. It is not a fixed property of an individual, but depends on the behavior of others and relationships with them. A major point of the review is the value of a comparative approach to the topic through the study of a diverse array of species, and in particular the value of studying teleost fishes, the most speciose groups of vertebrates. As the review emphasizes, teleosts show a wide variety of social systems. The sensitivity of their dominance relationships to social context and recent history show all the hallmarks of learning and cognition seen in birds and mammals, such as winner effects, bystander effects, audience effects, and dear enemy effects. Also fascinating is that teleosts underwent a whole genome duplication in their ancestry that makes them excellent candidates for gene manipulation approaches such as CRISPR/Cas9. The review will be valuable for a broad community of researchers interested in hormonal and neural mechanisms of social behavior.

Author Response: Thank you for the positive feedback and for your comments, which we address in greater detail below. We found these suggestions helpful and believe they will significantly improve the clarity of our manuscript.

1. There were a couple of puzzling omissions in the otherwise comprehensive coverage of the topic. One concerns 11-ketotestosterone. The authors should try to summarize whether, and if so, how, the role of 11-ketotestosterone differs from that of testosterone rather than leaving it up to the reader to try to figure this out from the table and text. Also, do teleosts have 11-ketotestosterone because of an enzyme gene duplication? Could its production conceivably be knocked out separately from that of other androgens? Also, at the end of section 3.1, where the role of estrogens in males is discussed, the authors should say something about estrogens and female status.

Author Response: Thank you for bringing these omissions to our attention, and these are all excellent points. 11-ketotestosterone (11-KT) is secreted by other vertebrates (e.g., mice: Yazawa et al. 2008, *Endocrinology*; humans: Imamichi et al. 2016, *J. Clin. Endocrinol. Metab.*), but circulating 11-KT levels are often low in most taxa, with the exception of fishes. We have clarified this point in Section 2.1 of our manuscript (page 4, lines 136-142). Prior work in teleosts suggests that circulating 11-KT and testosterone levels generally show similar patterns with respect to social status and behavioral interactions with conspecifics (e.g., aggressive encounters; Parikh et al. 2006, *Behav. Brain. Res.*; Taves et al. 2009, *Gen. Comp. Endocrinol*.), although to our knowledge, few studies have sought to distinguish the roles of these androgens in controlling social status. We have revised Section 3.1 so that it explicitly addresses this topic (page 7, lines 228-231), which should make this point clearer to readers. As the reviewer eludes to, 11-KT is primarily produced via its conversion from testosterone to 11β-hydroxytestosterone via the enzyme CYP11B1 and, subsequently, to 11-KT via the enzyme 11β-HSD2. Thus, 11-KT production is distinct from that of testosterone and can

be blocked experimentally to test how 11-KT and testosterone may regulate social status. We now discuss the aforementioned experiment as a potential future direction in Section 3.1 (page 7, lines 233-236). Regarding the role of estrogens in controlling social status in females, few studies have examined the neuroendocrine regulation of social rank in female teleost fishes. We present and discuss this point in Section 4 as a topic for future investigation (page 16, lines 516-525).

1. Also puzzling was the conclusion in the last sentence of section 3.2.1 (“and that androgen signaling and social opportunity combine to enable social ascent”). Doesn’t the result of the CA experiment argue against a role for androgen signaling in ascent?

Author Response: **Because subordinate male *A. burtoni* administered CA showed deficits in dominant-typical courtship behaviors following social ascent compared to control fish, we argue that these results support a role for androgens in regulating courtship, which is a critical behavior for both socially ascending and dominant males. We have included these additional details to further justify our conclusion (pages 12-13, lines 403-409).**

1. In Figure 1, the top right box shows a second gene duplication in the Salmoniformes. This was not mentioned in the figure caption. If it is discussed in the text, the authors could state where (which section) in the caption.

Author Response: **Thank you for pointing this out. There are a few orders of teleost fishes, such as the salmonids, that underwent an additional lineage-specific genome duplication, in addition to the teleost-specific whole genome duplication that occurred across all orders of teleosts. Due to the scope of our review, we have opted not to explicitly discuss this topic in our manuscript. We have, however, added a sentence to the legend for Figure 1 to clarify this point for readers (page 31, lines 1044-1046).**

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Author Response: **Thank you for highlighting these mistakes, which were an oversight on our part. We have carefully reread the manuscript to ensure that any remaining typos were revised accordingly.**

Competing Interests: No competing interests were disclosed.